INFECTION AND IMMUNITY, Aug. 1994, p. 3311–3319
0019-9567/94/$04.00+0

Vol. 62, No. 8

High-Molecular-Mass Lipopolysaccharides Are Involved in *Actinobacillus pleuropneumoniae* Adherence to Porcine Respiratory Tract Cells

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Received 27 December 1993/Returned for modification 9 February 1994/Accepted 10 May 1994

***Actinobacillus pleuropneumoniae* is the causative agent of porcine pleuropneumonia. The major adhesin of *A. pleuropneumoniae* has been identified as the lipopolysaccharides (LPSs) (M. Bélanger, D. Dubreuil, J. Harel, C. Girard, and M. Jacques, Infect. Immun. 58:3523–3530, 1990). Using immunoelectron microscopy and flow cytometry, we showed in the present study that LPSs were well exposed at the surface of this encapsulated microorganism. Immunolocalization with porcine lung and tracheal frozen sections showed that extracted LPS bound to the lung mesenchyme and vascular endothelium and to the tracheal epithelium, respectively. Inhibition of adherence of *A. pleuropneumoniae* with extracted LPS was also performed with lung and tracheal frozen sections. Acid hydrolysis of LPS revealed that the active component of LPS was not lipid A but the polysaccharides. LPSs from *A. pleuropneumoniae* serotypes 1 and 2 were separated by chromatography on Sephacryl S-300 SF, in the presence of sodium deoxycholate, according to their molecular masses. The adherence-inhibitory activity was found in the high-molecular-mass fractions. These high-molecular-mass fractions contained 2-keto-3-deoxyoctulosonic acid and neutral sugars, and they were recognized by a monoclonal antibody directed against *A. pleuropneumoniae* O antigen but not recognized by a monoclonal antibody against capsular antigen.**

Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia (28), a disease found worldwide that causes tremendous economic loss to the swine industry. Twelve serotypes of *A. pleuropneumoniae*, based on capsular antigens, have been recognized (29). Furthermore, the serotypes have different lipopolysaccharide (LPS) compositions, except that serotypes 1, 9, and 11, serotypes 3, 6, and 8, and serotypes 4 and 7 have common epitopes (31). In Québec, serotypes 1 and 5 are predominant, while serotype 2 is predominant in most European countries (27). The pathogenesis of porcine pleuropneumonia is not well understood. Several cytotoxic and hemolytic activities have been described (10–12, 22), but the virulence factors involved in colonization of the respiratory tract remain largely unknown.

The initial event in bacterial colonization is the adherence of microorganisms to the epithelial cells and/or mucus layer of the mucosal surfaces, which involves specific interactions between bacterial adhesins and host receptors (2, 30). We previously demonstrated that LPSs were the major adhesin of *A. pleuropneumoniae* involved in adherence to porcine tracheal rings maintained in culture (3). LPSs are complex molecules composed of three well-defined regions: the lipid A; the core, an oligosaccharide containing 2-keto-3-deoxyoctulosonic acid (KDO); and the O antigen, a chain of polysaccharides consisting of repeating units (18). Depending on the presence and the number of O-antigen repeating units, LPS can be rough, semirough (e.g., in *A. pleuropneumoniae* serotype 1), or smooth (e.g., in *A. pleuropneumoniae* serotype 2) (3, 7, 18). The purpose of the present study was to show the accessibility of LPS at the surface of this encapsulated microorganism, to localize the preferential binding sites of *A. pleuropneumoniae* LPS on porcine tissue sections, and to determine which region of this complex molecule is involved in adherence. We used strains of *A. pleuropneumoniae* showing two different LPS profiles, either semirough or smooth.

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MATERIALS AND METHODS

Bacterial isolates. *A. pleuropneumoniae* reference strains representing serotypes 1 (strain 4074) and 2 (strain 4226) were provided by A. Gunnarson, National Veterinary Institute, Uppsala, Sweden. A serotype 1 field isolate of *A. pleuropneumoniae* (FMV-87-682) was obtained from the clinical diagnostic laboratory, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada.

Growth conditions. Bacteria were grown on brain heart infusion (Difco Laboratories, Detroit, Mich.) agar plates supplemented with 40 μg of NAD per ml. Plates were incubated at 37°C in a 5% CO_2 atmosphere for 18 to 24 h.

Electron microscopy. (i) Immunolabeling. Bacteria were harvested in phosphate-buffered saline (PBS) (0.01 M, pH 7.4). Single drops of bacterial suspension were placed on Formvar-coated grids and were allowed to partially air dry. The grids were then placed sequentially on drops of PBS containing 1% (wt/vol) egg albumin (for 5 min) and mouse monoclonal antibodies against *A. pleuropneumoniae* serotype 1 O antigen (5.1 G8F10) or *A. pleuropneumoniae* serotype 2 O antigen (102-G02) (13) (for 30 min); both monoclonal antibodies were kindly supplied by Eva I. Stenbaek, Department of Biochemistry and Immunology, National Veterinary Laboratory, Copenhagen V, Denmark. The grids were then washed in distilled water and placed on drops of colloidal gold particles (10 nm) conjugated to goat anti-mouse immunoglobulin G

(IgG) (Sigma Chemical Co., St. Louis, Mo.) for 30 min. After a final wash in distilled water, the grids were stained with 0.2% (wt/vol) phosphotungstic acid (pH 7.1) and were examined with a Philips 201 electron microscope at an accelerating voltage of 60 kV.

(ii) LPS negative staining. LPS preparations were examined by negative staining. A drop of each LPS preparation, sonicated for 5 min, was placed on 200-mesh Formvar-coated grids. A drop of 1% (wt/vol) phosphotungstate was then applied to the grids, which were then examined as described above.

Flow cytometry. Washed overnight cultures of *A. pleuropneumoniae* were resuspended in PBS to an A_{540} of 0.2, equivalent to approximately 10^8 CFU/ml. Suspensions (1 ml) were centrifuged at 10,000 × *g* for 2 min, and the pellets were resuspended in 1 ml of a monoclonal antibody against serotype 1 or 2 O antigen and incubated for 60 min at room temperature. Samples were washed twice in PBS, and then 0.5 ml of sheep fluorescein isothiocyanate-conjugated anti-mouse IgG (Boehringer Mannheim, Laval, Québec, Canada) diluted 1:50 in PBS was added. After a further incubation of 60 min at room temperature, samples were washed twice in PBS and fixed with 2% paraformaldehyde. Cells were kept in the dark at 4°C until analyzed by flow cytometry. The flow analysis was performed with a FACStar flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) equipped with a water-cooled 2-W argon ion laser operating at 488 nm and a 200-mW light output. Multiparametric data were acquired for 10,000 events and analyzed by using Consort 30 software. The flow system was equipped with a 75-μm nozzle tip, and the analysis was performed at a flow rate of 500 events per s. Green (fluorescein isothiocyanate) fluorescence was collected in log scale, using a 530/30-nm filter. Data were analyzed in monoparametric histograms.

Extraction and isolation of LPSs. LPSs from *A. pleuropneumoniae* serotypes 1 and 2 were extracted and isolated by the method of Darveau and Hancock (8). Briefly, disrupted cells were treated with DNase, RNase, pronase, and sodium dodecyl sulfate (SDS) and were subjected to $MgCl_2$ precipitation and high-speed centrifugation. These LPS preparations contained less than 1% protein as determined by a dye-binding assay (Bio-Rad Laboratories, Richmond, Calif.), and no bands were detected after silver staining of SDS-polyacrylamide gels.

LPS hydrolysis. Ten milligrams (dry weight) of LPS was hydrolyzed at 100°C for 2 h in 1 ml of 1% (vol/vol) acetic acid previously saturated with nitrogen. Lipid A (insoluble) and polysaccharides (soluble) were separated by centrifugation at 12,000 × *g* for 10 min after neutralization with 5 N NaOH (1). Lipid A was washed and resolubilized in 1 ml of EDTA (20 mM) (26).

LPS fractionation by chromatography. LPSs of *A. pleuropneumoniae* serotypes 1 and 2 were separated in the presence of sodium deoxycholate by chromatography on a Sephacryl S-300SF (Pharmacia, Baie d'Urfé, Québec, Canada) column according to the procedure described by Peterson and McGroarty (33). Briefly, 20 mg of extracted LPS was resuspended in 3 ml of 0.25% (wt/vol) sodium deoxycholate–0.2 M NaCl–1 mM EDTA–10 mM Tris (pH 8.0), sonicated for 5 min, and subjected to gel filtration chromatography on a column (60 by 2.5 cm) of Sephacryl S-300SF. Elution was performed at a flow rate of 0.9 ml/min in deoxycholate-containing buffer. Fractions of 12 ml were collected. After extensive dialysis against column buffer (three times, 18 liters each) without deoxycholate at 37°C for 2 days and then against distilled water (three times, 18 liters each) at 4°C for another 2 days, the fractions were assayed for neutral sugar (9) and KDO (17) contents and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

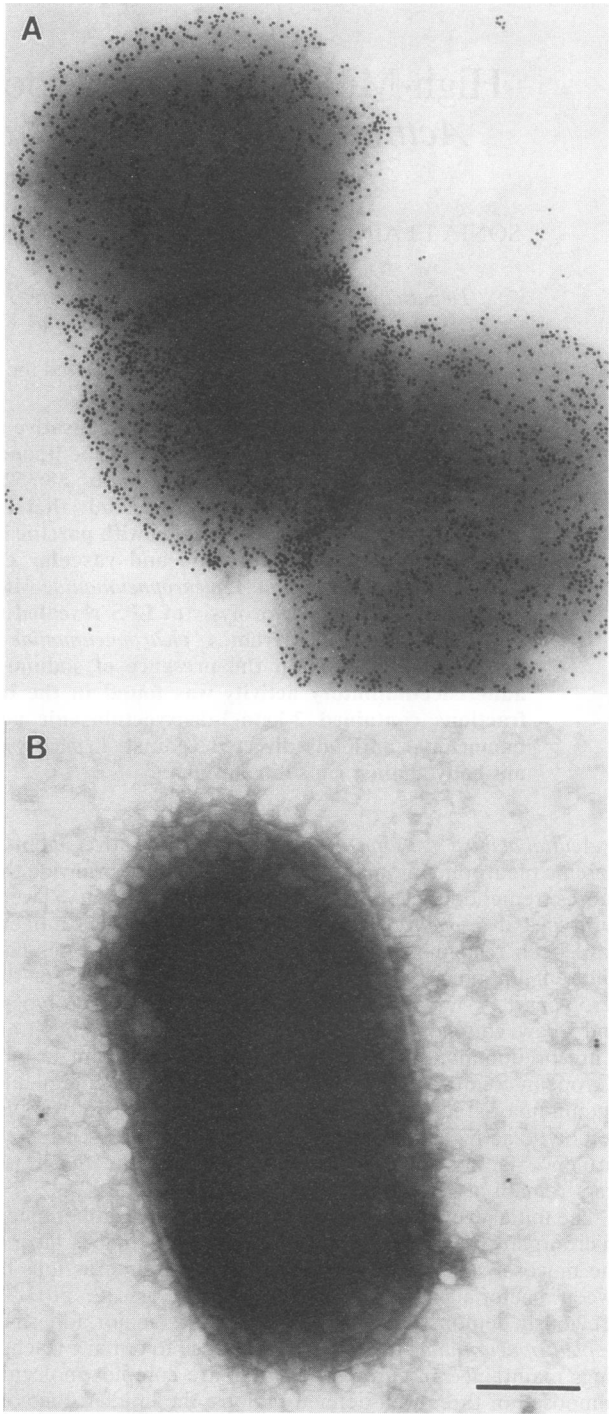

FIG. 1. Transmission electron micrographs of whole cells of an *A. pleuropneumoniae* serotype 1 isolate probed with monoclonal antibodies against serotype 1 (A) or 2 (B) O antigen and goat anti-mouse IgG–gold particles (10 nm). Bar, 200 nm.

LPS profiles. A volume of 500 μl of each fraction was lyophilized, suspended in 100 μl of solubilization buffer containing 10% (vol/vol) glycerol, 5% (vol/vol) β-mercaptoethanol, 2% (wt/vol) SDS, 0.0625 M Tris-hydrochloride (pH 6.8),

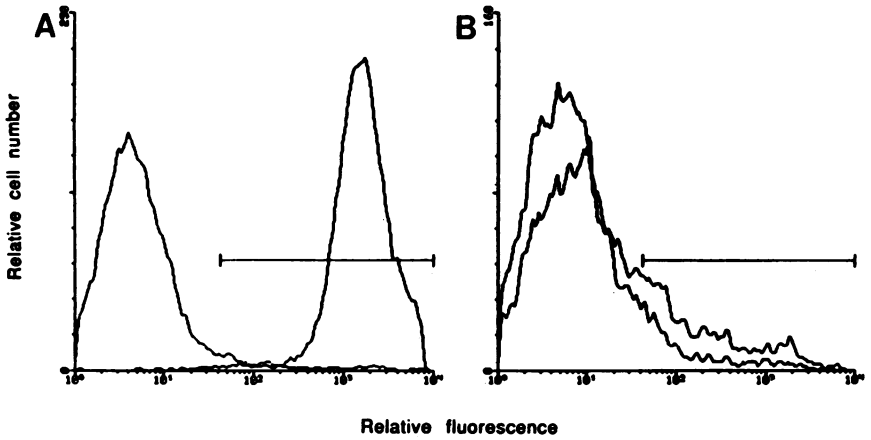

FIG. 2. Flow cytometry analysis of an *A. pleuropneumoniae* serotype 1 isolate. (A) Whole cells were labeled with the anti-mouse IgG fluorescein isothiocyanate (FITC)-conjugated antibody (left peak) and with monoclonal antibodies against serotype 1 O antigen plus the anti-mouse IgG FITC-conjugated antibody (right peak). (B) Whole cells were labeled with the anti-mouse IgG FITC-conjugated antibody and with monoclonal antibodies against serotype 2 O antigen plus the anti-mouse IgG FITC-conjugated antibody (left peaks). The horizontal bars indicate fluorescent cells.

and 0.025% (vol/vol) bromophenol blue, and heated for 20 min at 100°C. Volumes of 7 μl were loaded, and samples were separated by discontinuous SDS-PAGE by using a stacking gel of 4.5% (wt/vol) polyacrylamide and a separating gel of 15% (wt/vol) polyacrylamide (24). Samples were electrophoresed at 100 V (stacking gel) and 200 V (separating gel) with a Mini-Protean II apparatus (Bio-Rad). Gels were stained with the silver-staining procedure of Tsai and Frasch (36).

Dot blot. Ten-microliter aliquots of *A. pleuropneumoniae* serotype 1 LPS fractions were placed on a nitrocellulose membrane. All incubations were performed at room temperature and were followed by four 3-min washes with a Tris-saline buffer (10 mM Tris, 150 mM NaCl, pH 7.4). The membranes were first incubated for 1 h with 2% casein (a blocking solution) and then were incubated for 2 h with monoclonal antibodies against capsular polysaccharides of *A. pleuropneumoniae* serotype 1 (1.5 C5F4) or against LPS of *A. pleuropneumoniae* serotype 1. The membranes then were incubated for 1 h with a goat anti-mouse IgG (heavy plus light chains) horseradish peroxidase conjugate (Bio-Rad). Results of the reaction were revealed by addition of 4-chloro-1-naphthol and hydrogen peroxide (Sigma). As controls, bacterial suspensions of *A. pleuropneumoniae* serotypes 1 and 2 and extracted LPSs of both serotypes were dotted onto the membranes.

Frozen sections and adherence assay. Adherence to frozen sections was selected because we needed a system in which minute amounts of fractionated LPS could be tested for inhibitory activity. Lung and tracheal samples, obtained from newborn piglets, were washed in PBS, embedded in O.C.T. compound (Miles Laboratories, Inc., Elkhart, Ind.), frozen, and stored at −70°C until used (20). Frozen sections (4 to 6 μm thick) were cut in a cryostat microtome, mounted on glass slides, fixed in methanol for 1 min, and air dried (6). Bacteria were diluted in PBS containing 1% (wt/vol) bovine serum albumin and 0.01% (vol/vol) Tween 20 (PBS-BSA-T20) (37) to give an A_{540} of 0.2. A volume (100 μl) of the bacterial suspension was pipetted onto tissue sections on glass slides and incubated in a moist chamber at 37°C for 2 h. After intensive washing in distilled water, sections were stained with the Diff-Quik stain (Baxter Healthcare Corporation, McGraw Park, Ill.) according to the manufacturer's instructions. Upon microscopic examination, the number of bacterial cells attached to the tracheal epithelium was determined at a magnification of ×1,000.

Adherence inhibition assay. Frozen sections were preincubated with 25 μl of extracted LPS (2 mg/ml) and 75 μl of PBS-BSA-T20 in a moist chamber at 37°C for 30 min. Sections were then incubated with 25 μl of extracted LPS preparations and 75 μl of bacterial suspension in a moist chamber at 37°C for 2 h, washed, and stained as described above. Controls were made by substituting PBS-BSA-T20 for extracted LPS.

Localization of LPS-binding sites. A volume (100 μl) of extracted LPS (0.1 mg/ml) was deposited onto lung and tracheal frozen sections. The sections were incubated in a moist chamber at 37°C for 2 h and washed extensively. LPSs were detected by using either rabbit antisera raised against *A. pleuropneumoniae* serotypes 1 and 2 or monoclonal antibodies against *A. pleuropneumoniae* serotypes 1 and 2 and the AS/AP Immunostaining Kits (Bio/Can Scientific, Mississauga, Ontario, Canada). The slides were counterstained with Mayer's hematoxylin. Visualization of the preferential sites of attachment was done by microscopic examination at magnifications of ×100 and ×400. Controls were made either by substituting PBS-BSA-T20 for extracted LPS or by using normal rabbit serum or monoclonal antibodies against *A. pleuropneumoniae* serotype 1 capsular antigen.

RESULTS

Previous work in our laboratory identified LPS as the major adhesin of *A. pleuropneumoniae* involved in adherence to porcine upper respiratory tract cells. The aim of the present study was to show the accessibility of LPS at the surface of this encapsulated microorganism, to localize the preferential binding sites of *A. pleuropneumoniae* LPS on porcine lung and tracheal tissue sections, and to determine which region of this complex molecule is involved in adherence. We first determined the accessibility of LPS at the surface of this encapsulated microorganism, an essential prerequisite for a bacterial adhesin. Cells of *A. pleuropneumoniae* serotype 1 incubated with monoclonal antibodies against serotype 1 O antigen were heavily labeled with gold particles in immunoelectron microscopy (Fig. 1A), and were highly fluorescent when analyzed by

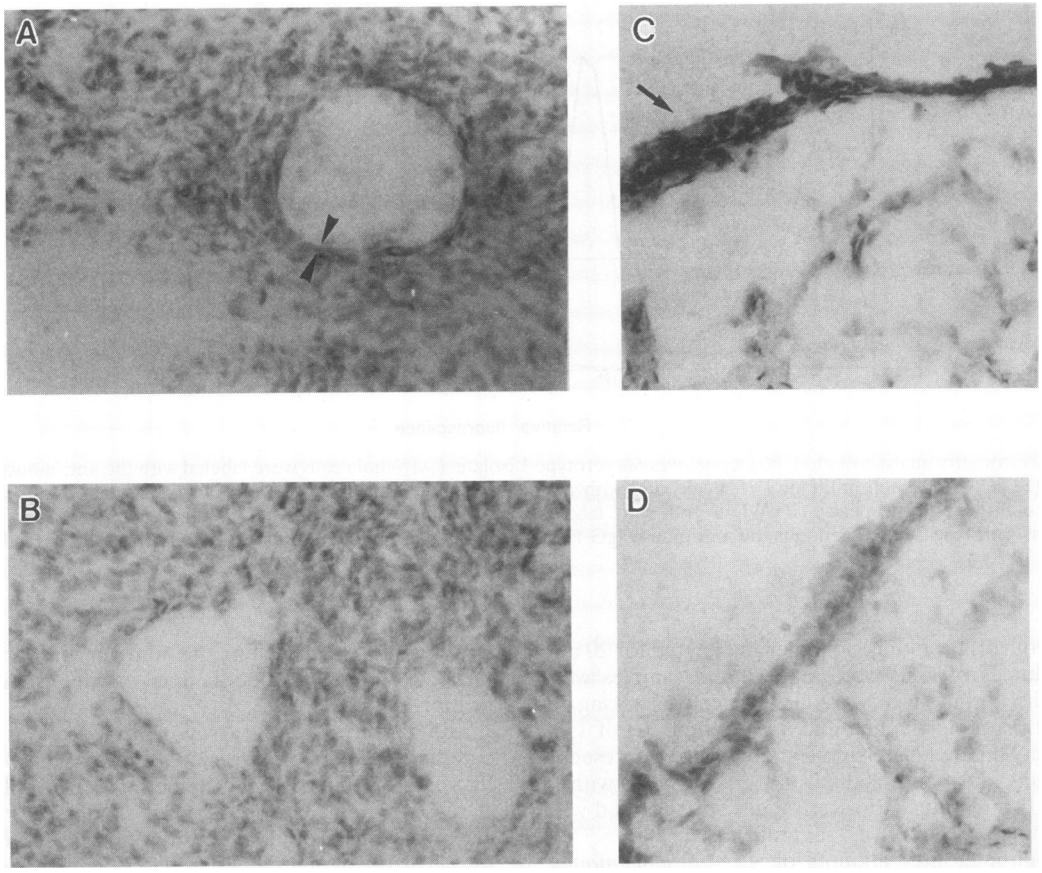

FIG. 3. Binding of *A. pleuropneumoniae* serotype 2 extracted LPS to porcine lung and tracheal frozen sections. Lung sections A and B (original magnification, ×250) and tracheal sections C and D (original magnification, ×400) were incubated with (A and C) or without (B and D) LPS. Sections were then incubated with a serotype-specific rabbit antiserum, and the results of the reaction were revealed with an alkaline phosphatase conjugate and a chromogenic substrate. Arrowheads and arrow denote areas of intense reaction.

flow cytometry (Fig. 2A), indicating that LPSs were surface exposed. Cells of *A. pleuropneumoniae* serotype 1 incubated with an irrelevant monoclonal antibody (against serotype 2 O antigen) were not labeled with gold particles (Fig. 1B) and were not fluorescent (Fig. 2B). It is important to note that when cells of *A. pleuropneumoniae* serotype 1 were incubated with monoclonal antibodies against serotype 1 capsular antigen, they were also heavily labeled with gold particles and highly fluorescent by flow cytometry, confirming the presence of capsular material on these cells. Similar results were obtained when cells of *A. pleuropneumoniae* serotype 2 were incubated with monoclonal antibodies against serotype 2 O antigen (data not shown).

Porcine lung and tracheal frozen sections were incubated in the presence of either *A. pleuropneumoniae* serotype 1 or 2 extracted LPS. Detection of bound LPS was done by immunostaining, using a serotype-specific rabbit antiserum or monoclonal antibodies directed against *A. pleuropneumoniae* serotype 1 or 2 O antigen, an alkaline phosphatase conjugate, and a chromogenic substrate. Microscopic examination showed an important brown deposit localized at the lung vascular endothelium (Fig. 3A, arrowheads) and the tracheal epithelium (Fig. 3C, arrow). In addition, the lung mesenchyme showed diffuse staining of a lesser intensity. Controls in which LPSs were omitted (Fig. 3B and D) did not show any reaction.

Inhibition of adherence of *A. pleuropneumoniae* serotypes 1 and 2 to tracheal frozen sections was observed with extracted LPS (Fig. 4). In order to determine which region of the LPS molecule was essential for inhibition of adherence, extracted LPSs were hydrolyzed. After acid hydrolysis of *A. pleuropneumoniae* serotype 1 and 2 extracted LPSs, the inhibiting activity

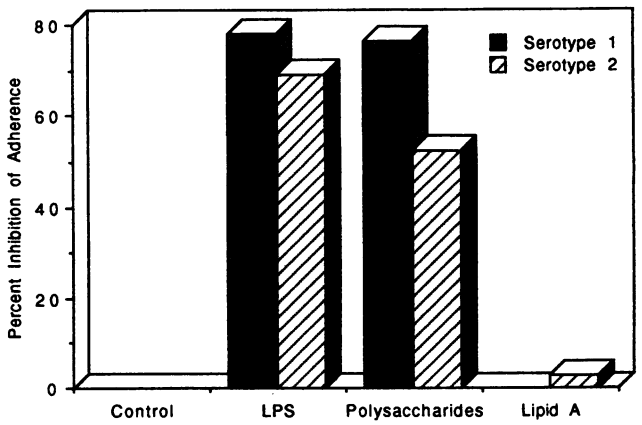

FIG. 4. Inhibition of adherence of an *A. pleuropneumoniae* serotype 1 isolate and a serotype 2 reference strain to porcine tracheal frozen sections by homologous polysaccharides and lipid A obtained after acid hydrolysis of *A. pleuropneumoniae* extracted LPS.

was associated with the polysaccharide moiety of LPS, while the resolubilized lipid A was totally devoid of activity (Fig. 4).

We then separated LPS molecules according to their molecular masses by chromatography in the presence of deoxycholate. *A. pleuropneumoniae* fractionated LPSs were divided into five major pools that each contained KDO and neutral sugars (Fig. 5). Silver staining of these fractionated LPSs after SDS-PAGE revealed molecules of decreasing molecular masses (Fig. 6). Material from pool A of serotype 1 and 2 LPSs remained at the top of the polyacrylamide gel. Pools B, C, and D were composed of LPSs with decreasing chain lengths, whereas pool E contained LPS molecules with no or small numbers of O-antigen repeating units (Fig. 6). Dot blotting was used to ascertain the LPS nature of the different pools of LPS. *A. pleuropneumoniae* serotype 1 extracted LPS and all fractions reacted with the monoclonal antibody directed against *A. pleuropneumoniae* serotype 1 O antigen and not with the monoclonal antibody directed against *A. pleuropneumoniae* serotype 1 capsular polysaccharides.

Inhibition of adherence was then done with the different pools. Pool A, containing the high-molecular-mass fractions of *A. pleuropneumoniae* serotype 1 LPS, clearly showed more inhibition activity than the other pools (Fig. 7), an activity comparable to that obtained with control, unfractionated LPS. Similar results were obtained with fractions of *A. pleuropneumoniae* serotype 2 LPS (data not shown).

Finally, fractionated LPSs were examined by transmission electron microscopy after negative staining. Molecular aggregates of extracted LPS as well as fractions corresponding to pools B to D appeared as ribbons approximately 12 nm wide (Fig. 8A and C). High-molecular-mass fractions of pool A contained thin filaments approximately 2 nm wide (Fig. 8B), whereas vesicles 55 to 75 nm in diameter were observed in the low-molecular-mass fractions of pool E (Fig. 8D).

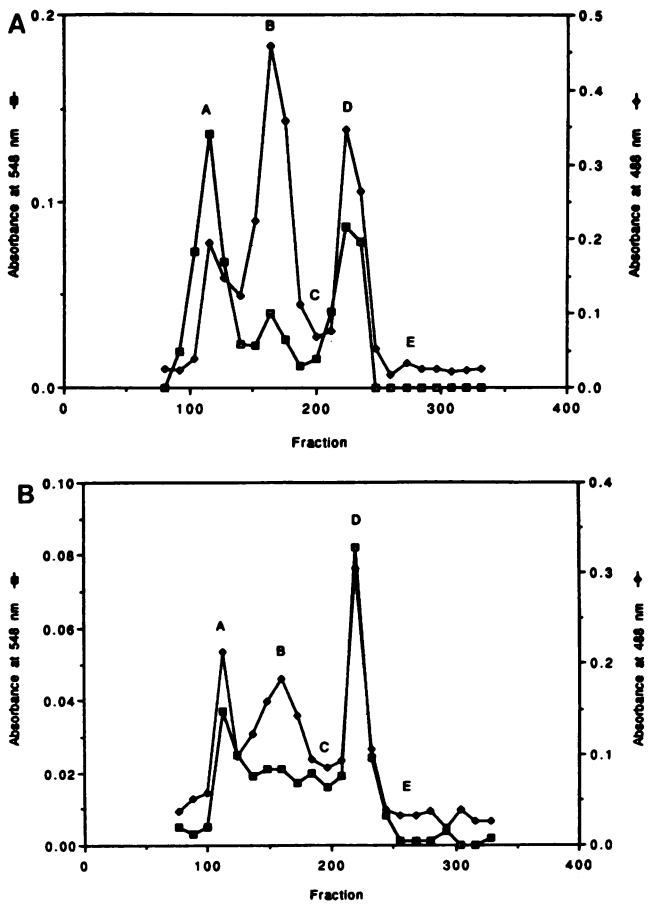

FIG. 5. Fractionation of extracted LPS from *A. pleuropneumoniae* serotype 1 (A) or serotype 2 (B) on a Sephacryl S-300SF column in the presence of 0.25% deoxycholate. Fractions (milliliters) were analyzed for KDO content (squares) and for neutral sugar content (diamonds). Fractions were grouped into five pools (A to E).

DISCUSSION

The initiating event in the pathogenesis of most bacterial pulmonary infections is most probably the establishment of the organisms in the upper respiratory tract. Adherence is a complex interaction between the bacterium and the target cell which enables colonization to occur and allows the bacterium to exert its pathogenic and immunogenic effects. We have previously shown the involvement of LPS in adherence of *A. pleuropneumoniae* to porcine respiratory tract cells and mucus (3, 4). Cells of *A. pleuropneumoniae* serotype 1 and 2 reference strains have been shown to be covered by a capsule layer of approximately 220 and 85 nm, respectively, as determined by electron microscopy after immunostabilization (21). The results of the present study, using immunoelectron microscopy and flow cytometry, indicate that LPSs are indeed accessible at the surface of this encapsulated microorganism, which is an essential prerequisite for any bacterial adhesin. Surface exposure of LPS has been reported for other heavily encapsulated organisms, including *Klebsiella pneumoniae* (35).

We used frozen sections to further study the involvement of LPS in the adherence of *A. pleuropneumoniae* to porcine respiratory tract cells. This adhesion assay combined with immunostaining showed that *A. pleuropneumoniae* extracted LPS adhered to the vascular endothelium and the mesenchyme of porcine lung and to the tracheal epithelium. These observations support the idea that *A. pleuropneumoniae* LPSs play an important role in adherence. Our in vitro model for adhesion of *A. pleuropneumoniae* with tracheal frozen sections was more convenient for the quantitative evaluation of attached bacteria than that with lung frozen sections because it allowed us to count bacterial cells over a definite area (i.e., the epithelial surface). Adherence inhibition experiments showed that extracted LPSs were able to prevent the adherence of *A. pleuropneumoniae*, which confirmed our previous work with porcine tracheal rings maintained in culture (3).

Acid hydrolysis of LPS clearly indicated that the polysaccharides were the essential LPS components responsible for adherence inhibition, since the lipid A by itself was not able to prevent attachment of *A. pleuropneumoniae*. Most biological effects of LPS are associated with lipid A (5, 34). It has been established that molecular structures present in endotoxin specifically recognized by different cells may be located in the hydrophobic (lipid A) or in the hydrophilic (polysaccharide) regions of the macromolecule (15). Although most LPS receptors are known to recognize the lipid A (15, 38), a lectin-like binding site for endotoxin has been identified on activated T cells (25) and on macrophages (14, 16). We do not know at this time what are the cellular receptors for *A. pleuropneumoniae* LPS.

Gel chromatography on Sephacryl S-300SF identified the high-molecular-mass fractions as the most effective molecules in adherence inhibition. These high-molecular-mass fractions were not detected by SDS-PAGE and silver staining. However, the KDO and Dubois assays revealed the presence of KDO and neutral sugars, as for the other fractions. Furthermore,

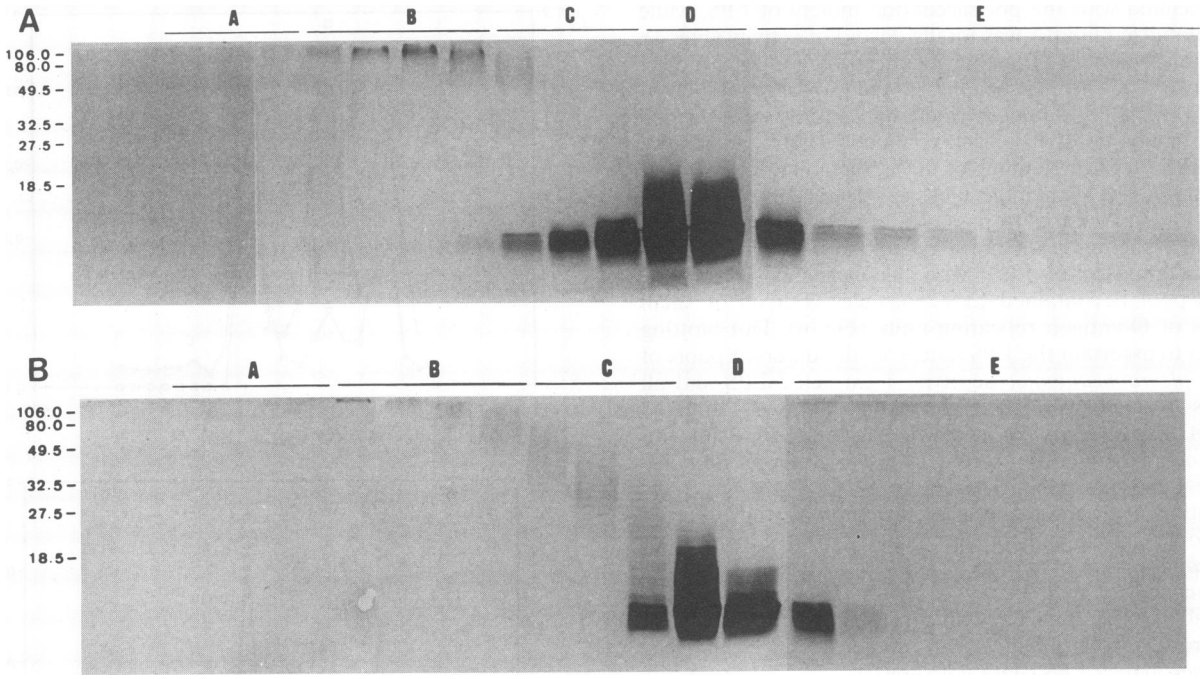

FIG. 6. Silver-stained SDS-PAGE profiles of *A. pleuropneumoniae* serotype 1 (A) or serotype 2 (B) extracted LPS fractionated on a gel filtration column. Pools of fractions (A to E) are as in Fig. 5. The positions of low-molecular-mass markers (in kilodaltons) are indicated on the left.

monoclonal antibodies against *A. pleuropneumoniae* O antigen reacted with all these fractions. The lack of silver-stained bands in the higher-molecular-mass fractions may be due to the inability of the material to penetrate in the separating gel and/or to the absence of reactive groups involved in the staining reaction, as reported by Peterson and McGroarty, who observed the same phenomenon with *Escherichia coli* O111:B4 LPS (33). Interestingly, the gel filtration chromatography demonstrated that *A. pleuropneumoniae* serotype 2 smooth LPS is composed of O-antigen repeating units distributed more uniformly than the O-antigen repeating units of *A. pleuropneumoniae* serotype 1 semirough LPS. SDS-PAGE profiles showed a very constant decrease in molecular mass for *A. pleuropneumoniae* serotype 2 fractionated LPS. This was not observed for *A. pleuropneumoniae* serotype 1 fractionated LPS, in which the distribution of O-chain lengths was bimodal.

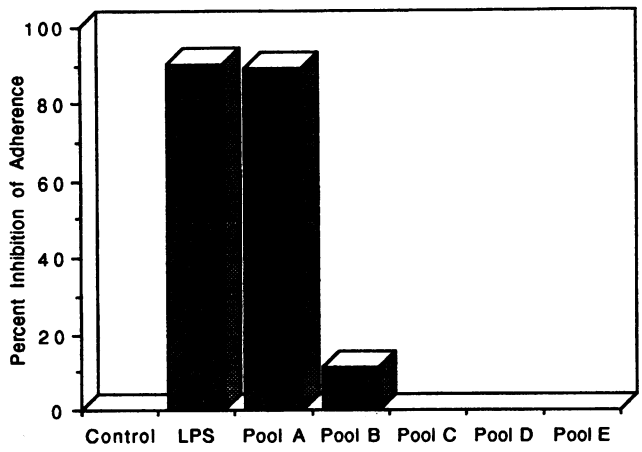

FIG. 7. Inhibition of adherence of an *A. pleuropneumoniae* serotype 1 isolate to porcine tracheal frozen sections by pooled fractions obtained after gel filtration chromatography of extracted LPS from *A. pleuropneumoniae* serotype 1.

The electron microscopic examination showed a filamentous form for the higher-molecular-mass fractions of LPS. These filaments had a smaller diameter and longer length than the ribbons observed in the extracted LPS preparation and did not adopt a vesicle conformation as observed with the lower-molecular-mass fractions. Kato (23) reported that negatively stained smooth LPSs from *K. pneumoniae* O3, *E. coli* O9 and O127, and *Salmonella minnesota* have common structural features consisting principally of ribbon-like structures, which branch freely and often form loops, and of spheres; both structures are covered with fine hairy structures. Kato also suggested that the surface projections covering the ribbon-like structures and the spherical structures would be polysaccharides. These observations suggest that LPS aggregates adopt different shapes depending on the O-antigen chain length and the lipid content. Similar results with LPS of *E. coli* O111:B4 have been described (32).

Fractionation of *A. pleuropneumoniae* LPS showed that low-molecular-mass fractions corresponding to the core-lipid A region did not inhibit adherence but that the high-molecular-mass fractions were responsible for adherence inhibition. This was observed with the semirough (serotype 1) as well as the smooth (serotype 2) LPSs of *A. pleuropneumoniae*. Our results suggest that longer polysaccharide chains embodied the adhesin and that these longer chains, possibly because of their conformation, are more able to efficiently inhibit adherence. Interestingly, we have shown in a previous study that adherence of *A. pleuropneumoniae* isolates to porcine tracheal rings was related to their LPS profile; isolates with a smooth profile adhered in higher numbers than isolates with a semirough profile (3). Taken together, our results clearly suggest that

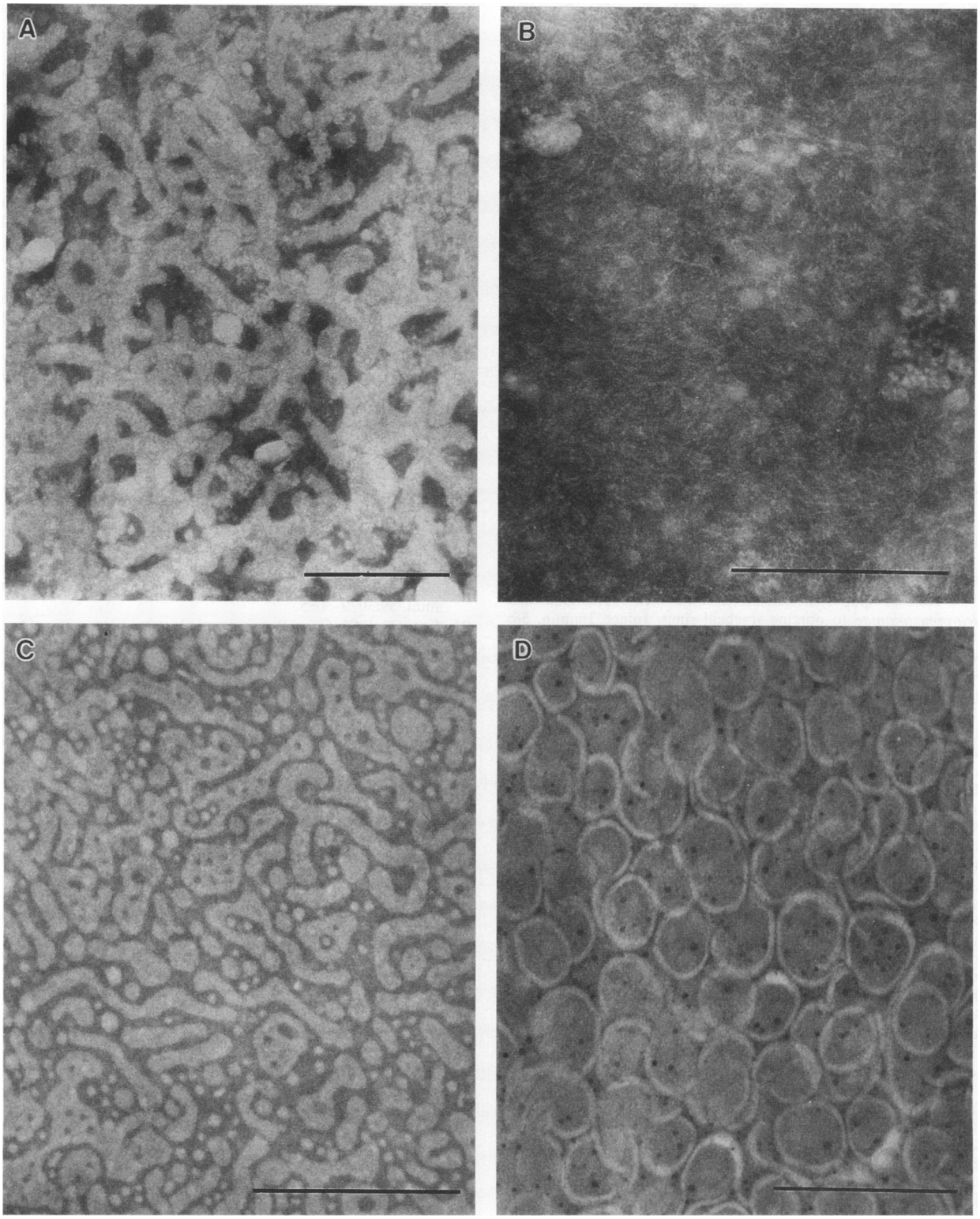

FIG. 8. Transmission electron micrographs of negatively stained *A. pleuropneumoniae* serotype 2 LPS. (A) Extracted LPS preparation. (B to D) LPS preparations obtained after chromatography on a Sephacryl S-300SF column. (B) Pool A; (C) pool C; (D) pool E. Bars, 200 nm.

long-chain LPSs play a predominant role in adherence of *A. pleuropneumoniae*. Since many bacterial adhesins also act as biological effector molecules (19), eukaryotic cells would be expected to experience the well-known effects of endotoxin in conjunction with the presentation of an LPS adhesin. Further studies are needed to identify the cellular receptors and to determine whether *A. pleuropneumoniae* LPS does elicit a response in the target cells.

ACKNOWLEDGMENTS

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC A3428 to M.J.), from Association Pulmonaire du Québec, and from a research partnership program involving the Natural Sciences and Engineering Research Council of Canada, Agriculture Canada, and Fédération des Producteurs de Porcs du Québec. Sonia-Élaine Paradis is the recipient of a studentship from Fonds pour la Formation de Chercheurs et l'Aide à la Recherche.

We are grateful to Bernadette Foiry for performing the electron microscopy, to Serge Sénéchal for his expertise in flow cytometry, and to Caroline Bégin for technical assistance.

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